FIREFLY LUCIFERASE ASSAY

This protocol is adapted from Promega's Luciferase Assay System Protocol by the Gene Expression Lab.
This protocol is for use with Promega's Luciferase Assay Systems. For additional technical inquiries, contact Technical Service at 800-356-9526 or www.promega.com

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BEFORE STARTING THE EXPERIMENT

1. Prepare the Firefly Luciferase Assay Reagent. Add Luciferase Assay Buffer (10 mL) to the vial containing the lyophilized Luciferase Assay Substrate.
    a. To avoid exposure of the Luciferase Assay Reagent to multiple freeze-thaw cycles, aliquot the reconstituted reagent into working aliquots of 1 mL and store any unused reagent at -70˚C.
2. Prepare the Reporter Lysis Buffer (RLB)
    a. Reporter Lysis Buffer is supplied as a 5X concentrate. Prepare a sufficient quantity of the 1X working solution by adding 1 volume of 5X Reporter Lysis Buffer to 4 volumes of distilled water and mixing well.
    b. The diluted (1X) RLB may be stored at 4°C for up to one month. However, we recommend that Lysis Buffer be prepared fresh in the amount needed for each experiment. Store the 5X Reporter Lysis Buffer at –20°C.

Preparing Cell Lysates:

1. Carefully remove the growth medium from cells to be assayed. Rinse cells with PBS, being careful to not dislodge attached cells. Remove as much of the PBS rinse as possible.
2. Add enough 1X lysis buffer (CCLR, RLB or PLB) to cover the cells (e.g., 400 µL/60mm culture dish, 900µl/100mm culture dish or 20 µL per well of a 96-well plate). If using RLB, perform a single freeze-thaw to ensure complete lysis.
3. Rock culture dishes several times to ensure complete coverage of the cells with lysis buffer. Scrape attached cells from the dish. Transfer cells and all liquid to a microcentrifuge tube. Place the tube on ice.

4. Vortex the microcentrifuge tube 10-15 seconds, then centrifuge at 12,000×*g* for 15 seconds (at room temperature) or up to 2 minutes (at 4°C).
5. Transfer the supernatant to a new tube.
6. Store the supernatant/cell lysate at -70°C or proceed to Firefly Luciferase Assay.

Protocol for Manual Luminometers

1. Dispense 100 µL of the Luciferase Assay Reagent into luminometer tubes, one tube per sample.
2. Program the luminometer to perform a 2-second measurement delay followed by a 10-second measurement read for luciferase activity. The read time may be shortened if sufficient light is produced.

Note: When using shorter assay times, validate the luminometer over that time period to ensure that readings are taken at a flat portion of the signal curve.

3. Add 20 µL of cell lysate to a luminometer tube containing the Luciferase Assay Reagent. Mix by pipetting 2-3 times or vortex briefly.
4. Place the tube in the luminometer and initiate reading.
5. If the luminometer is not connected to a printer or computer, record the reading.